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Diagnosis of the Dried Powder of Liana Bark *Landolphiaowariensis* P. Beauv. (Apocynaceae)

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Abstract: ***The diagnosis of powdered liana bark from Landolphiaowariensis (Apocynaceae). This is a micrographic and granulometric study of the powder. The organoleptic parameters have been appreciated. Elements were identified under an optical microscope. The particle size distribution was carried out by sieving and laser microscopy. Characteristic elements of the powder have various geometric shapes. The sizes vary from 0.3 to 0.35µm. The powder is fine. The waist line looks like a bell. The mean, mode and median coincide on the same size. Thus the distribution is normal, the studied powder is homogeneous, not adulterated.***

Keywords: *Landolphiaowariensis*, micrograph, granulometry

1. Introduction

Traditional medicine constitutes a major aspect of cultural heritage in Africa. The African population is still attached to this medicine. According to the World Health Organization (WHO), nearly 80% of this population use traditional medicine for their health needs [1]. It is imperative to evaluate the clinical efficacy, to ensure the quality and to research the harmlessness of medicinal plant drugs, to guarantee sufficient follow-up of patients. Indeed, several studies carried out on traditional treatments from plants have reported a problem of toxicity [2]. Also powder mixes, contaminations, fraud on the type and origin of powders constitute a major problem often responsible for toxicity or ineffectiveness leading to falsemedication.

In this research work, we carried out work upstream of the design of an improved traditional drug. This work focused on the micrographic study and the granulometry of the bark powder. It seemed interesting to us tocarry out this study in order to know the characteristic elements of the powder of the bark of the trunk of *Landolphiaowariensis.* This research work will ensure an effective formulation, safety and exploration of this drug ofmajor interest for the treatment of hemorrhoidal crises.

2. Documentary Investigation

Micrography allows the identification of the characteristic elements and organoleptic parameters (taste, smell, color, appearance) of a powder. The particle size provides information on the size and the statistical distribution of the sizes of the powder. It has a direct impact on the stability, the homogeneity of the formulations chosen and the bioavailability of the active ingredients obtained from the powders. The rheology, segregation, flocculation, agglomeration of the elements of aformulation, depend on the particle size of the drug [3]. *Landolphiaowariensis* P. Beauv. (BrienMalinké) is a plant species used by the peoples of northern Côte d'Ivoire for the treatment of hemorrhoidal crises, sprains, pain and dizziness. This plant is a savannah shrub. Also, it can also be found in forest where, it becomes an enormous climbing plant, producing stems and being able to reach a length of 100 meters or more with a basal diameter of 30 to 40 cm. The fruit, edible, is commonly picked from the wild and is highly valued [4].

3. Materials and Methods

3.1 Equipment

3.1.1 Plantmaterial

The study focused on the trunk bark of liana *Landolphiaowariensis (*Apocynaceae).

3.1.2 Laboratory equipment

The laboratory equipment was made up, among other things, of a Retsch type mill **.** SM200, an optical microscope of the optika type attached to a laptop computer of the Dell type, a micrometric slide and a set of sieves numbered from 1 to 6 and the mesh of which is respectively 1.4 mm; 500 µm; 355µm; 150 µm; 100 µm; 50 µm and the cover.

3.2 Methods

3.2.1 Sample processing

The drug consisting of trunk bark was harvested manually, using a machete, in November 2017, in Djibrosso in the department of Kani, north-western Côte d'Ivoire. This drug was then washed quickly with distilled water and then dried at room temperature for 15 days. Part of this unwashed bark,

associated with the leaves of the plant, made it possible to identify the species under herbarium n° 147 by Professor Tra Bi Fesan Honora, Botanist, at Nangui Abrogoua University. This dried trunk bark of *Landolphiaowariensis* was pulverized thanks to the crusher. The powder obtained was placed in hermetically sealed vials and stored at room temperature at 25 ° C. This powder was used to carry out the micrography and the particle size analysis.

3.2.2 Botanical study of the drug

This study has 3 parts.

- **Macroscopic study of the plant and thedrug**

It was carried out by observing the plant species and the organ used which is the bark of the trunk of the liana.
This naked eye examination made it possible to describe the plant and the dried drug.

- **Study of the organoleptic characteristics of drugpowder**

Analysis of these characteristics focused on the appearance, smell, taste, and color of the drug sprayed. Indeed, the determination of the appearance was carried out by touching the powderto check for grainy or amorphous character. To characterize the odor, 1 mg of powder was taken between thumb and forefinger. The odoriferous constituents are released slowly and have been tested several times. The intensity of the odor was first noted by the following parameters: “absent, weak, strong and very strong” Then, the type of odor was determined: “Aromatic, fruity or characteristic”. Regarding taste, 5 grams of drug powder was placed and kept in the mouth without swallowing, for 10 to 30 seconds. The sample was then spat out and the mouth rinsed. The taste was appreciated and rated: “Spicy, Bland, Sour, Bitter, Sweet, Salty, Hot. Finally the color was declined after observation with the naked eye [5].

- **Micrographic study of liana bark powder *Landolphiaowariensis***

The micrography consisted in observing under an optical microscope, between slide and coverslip, the bark powder of *Landolphiaowariensis.* To do this, a drop of aqueous KOH (5%) was placed on a clean slide then sprinkled with the drug spray. Then, this preparation was covered with a coverslip before being observed under a microscope at magnifications 4 then 10. The Gx40 magnification enabled us to refine the details of the elements observed. This study revealed fragments of plant tissue and crystals [11]

3.2.3 Particle size study of thepowder

This study which allows to know the shape and size of the particles was carried out in 2 stages.

- **Characterization of particle shape by sieving**

The powder obtained after grinding was sieved. This technique consisted in passing 100 grams of drug powder through 6 different successive sieves of decreasing mesh size. These were subjected to calibrated vibrations for 15 minutes according to the method described by the European Pharmacopoeia 10th edition [6]. Indeed, the sieves were arranged in decreasing order of mesh size as follows: the cover covering the sieve 1 (1 mm), sieve 2 (600 µm), sieve 3 (300 µm), sieve 4 (200 µm), sieve 5 (100 µm) then sieve 6 (50 µm). Each fraction of powder obtained on each sieve was weighed in order to determine the percentage of mass and then to describe the size and size of theparticles.

The results obtained are expressed as a percentage by weight. These are obtained by making the ratio of the weight of the drug collected over the total weight of the initial drug, the whole being multiplied by one hundred. The fractions are divided into different types of powder:

- Coarse powder> 95% passes through the 1.4 mm sieve and <40% passes through the 355 µm sieve
- Moderately fine powder: >95%passesthroughthe355µm sieve and<40% passes through the180µm sieve
- Fine powder> 95% passes through the 180 µm sieve and <40% passes through the 125 µm sieve
- Very fine powder> 95% passes through the 125 µm sieve and <40% passes through the 90 µm sieve

After sieving, a histogram and a frequency curve were carried out.

- **Determination of particle size bymicroscopy**

This characterization of the particles was carried out under certain conditions such as individuality, visibility, immobility, homogeneity of the size or orientation of the particles and the absence of foreign structure [7].
In practice, a pinch of very fine drug powder is deposited on the micrometric slide, under the eye piece of the microscope coupled with image analysis software for direct observation of the shapes and the measurement of the size of the particles according to the technique of Feret. This technique measures the distance between two tangents parallel to opposite sides of the particle which is the Feret Diameter. The measurement concerned 400 particles.

4. Results and Discussion

4.1 Botanical Aspect

Macroscopic drug identification

Landolphiaowariensis, P. Beauv., 1805, is a liana of the Apocynaceae family. This species, found in the north-west of the Ivory Coast, in the tropics, is very large and measures more than 10 meters long. This liana has leaves that grow in pairs and are elliptical acuminate. The inflorescences are terminal. The flowers are small, white and clustered in small terminal panicles. This liana branches out several times. Also she wears powerful tendrils which often strangulation. The bark that constitutes the drug is rough, dark brown or greyish brown with patches of grayish colors(Figure1).

Figure 1: Curved Shape of *Landolphiaowariensis*

To harvest the drug, the vine is cut into pieces and then dried. This bark in the dry form is reddish-brown or dark gray-brown in color, is rougher, and still bears the constrictions of its powerful tendrils (Figure 2).

Figure 2: Dried Pieces of *Landolphiaowariensis*

The dried bark, detached from the wood, is in the form of a more or less curved piece of pipe. The color is, on the outside, dark brown mottled with greyish color and on the inside, whitish (Figure 3).

Figure 3: dried bark of *Landolphiaowariensis*

Organoleptic characteristics of the drugpowder

The observation of the powder *L. owariensis* allowed to see a powder of granular appearance of light brown color after grinding. This color gradually turns, after 3 hours, to dark brown comparable to dark chocolate (Figure 4). The smell is characteristic aromatic and very weak. The latter gradually disappears. The drug powder has a slightly bitter and astringent taste.

Figure 3: bark powder *Landolphiaowariensis*
A: light brown color; B: dark brown color

Drug Micrograph

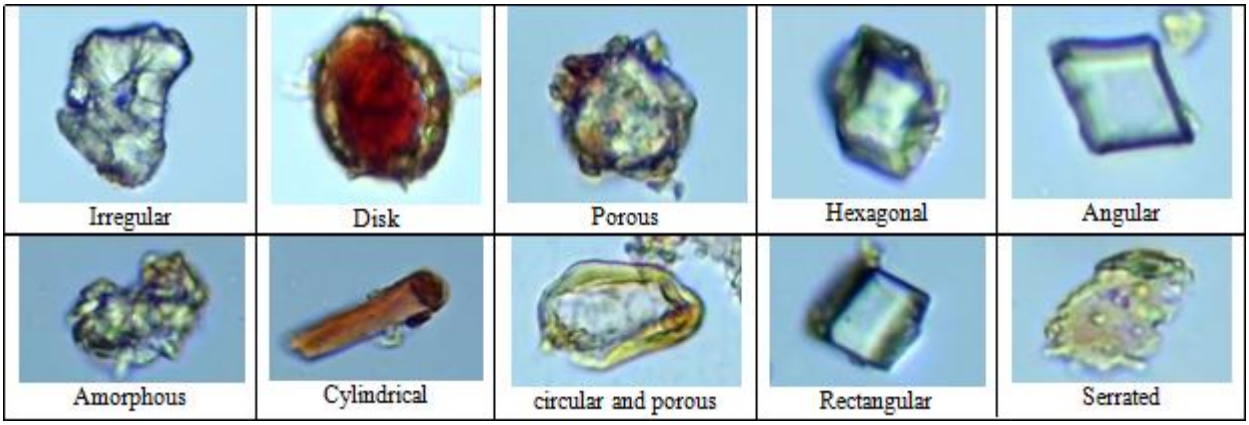

Figure 5: different geometric shapes of particles (G x 40)

The micrography made it possible to observe several particles of variable shape (Figure 5) which are among others circular shapes, rectangular shapes, cylindrical shapes and tetrahedral angular shapes.

Among these particles observed, the characteristic elements of the powder (Figure 6) were particularly observed:

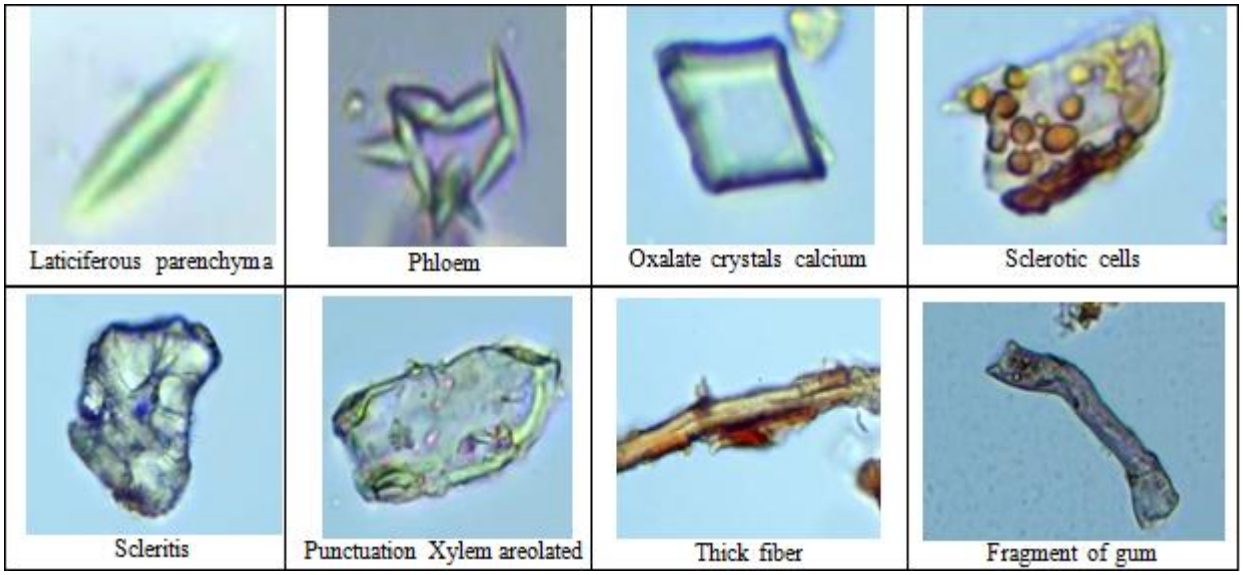

Figure 4: characteristic elements of liana bark powder *Landolfiaowariensis* (Gx40)

The character-defining elements of the bark powder of the liana ***L. owariensis*** are among others the raphids, the prisms of calcium oxalates and the laticiferous parenchyma.

Particle size study

Result of particle size measurements bysieving

Table 1: Sieve Sizes and particle masses of Landolfiaowariensis powder

Mesh sizes (sieve)	Mass of empty sieves (g)	Mass of sieves after sieving (g)	Powder mass (g)	% mass floor
1 mm	239.4	241.57	2, 17	2.17%
600 µm	280.2	284.73	4.53	4.53%
300 µm	242.1	248.9	6.8	6.80%
200 µm	262.3	273.11	10, 81	10.81%
100 µm	239.5	251.7	12.2	12.20%
50 µm	282.6	297.86	15.26	15.26%
Lower tray	**350.2**	**398.43**	**48.23**	**48.23%**

By sieving, almost half of the powders are found in the lower sieve tank of 50µm (48.23% °). Our powder is fine.

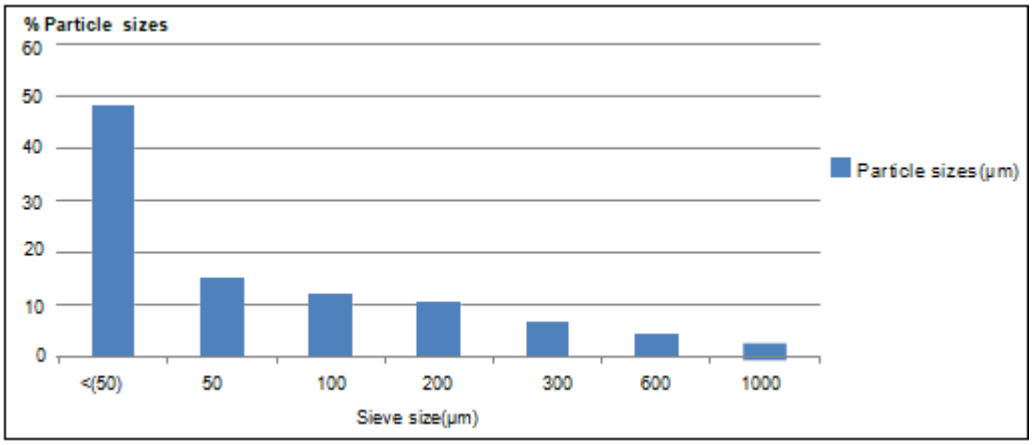

Figure 7: Percentage curve of particle sizes as a function of sieve sizes

By opticalmicroscopy

Table 2: Particle Sizes of the powder Landolfiaowariensis (Apocynaceae)

Sizes **µm**	0.15-0.2	0.2-0.25	0.25-0.3	0.3-0.35	0.35 - 0, 4	0.4-0.45	0.45 -0.5	0.5-0.55	055 -0.6	0.6-0.65
Workforce	1	11	47	110	70	51	3	5	0	2
Class Center **µm**	0.175	0.225	0.275	0.325	0.375	0.425	0.475	0.525	0.575	0.625
Frequencies	0.003	0.175	0.156	0.366	0.233	0.17	0.01	0.016	0	0.007
Frequencies cumulative	0.003	0.039	0.195	0.561	0.794	0.964	0.974	0.99	0.99	0.997
Frequencies%	0.3	3.6	15.6	36.6	23.3	17	1	1.6	0	0.7
Frequencies% Cumulative	0.3	3.9	19.5	56.1	79.4	96.4	97.4	99	99	99.7

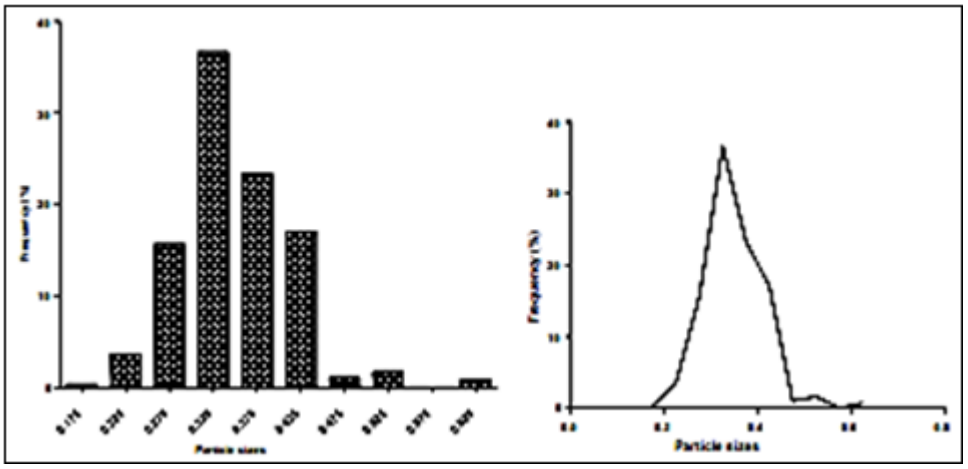

Figure 8: Size curve and histogram of the particle sizes of the powder *L.owariensis (*50 µm mesh sieve)

The size curve has a bell shape. This demonstrates the homogeneity of the powder. Indeed, the size of the powder is homogeneous. It depends on spraying anddrying.

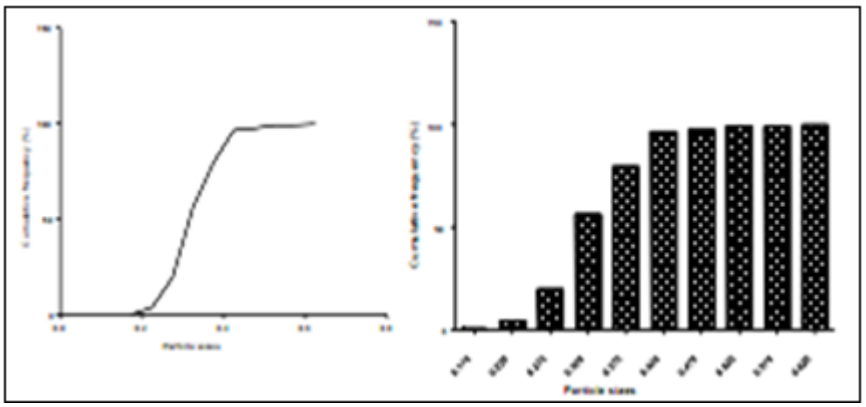

Figure 9: Curve and histogram of the cumulative frequency of the sizes of the particles of the *L. owariensis(50)* µm mesh sieve)

The cumulative frequency curve of the sizes at a plateau pace. This confirms the homogeneity of the powder. The distribution of the powder is normal and homogeneous. The powders are not contaminated.

3. Discussion

*Landolphiaowariensis*is an Apocynaceae used in several regions of black Africa for its various therapeutic virtues, including anti-hemorrhoidal activity. Our work has enabled us to highlight the botanical characteristics of the powder a through micrographic and granulometric studies. Thus, according to the studies carried out, different constituents and particle size parameters have been clarified:

- The cumulative frequency curve gives a plateau look. The curves are not sheared. They confirm more than the powder of the bark of liana of *L .owariensis*is homogeneous.
- The mode (0.3) µm between the interval (0.3 - 0.35) µm is the most common diameter in a distribution. It corresponds to the maximum of the frequency curve. The median (0.35) µm between (0.3 - 0.35) µm, represents the value for which an identical number of particles is above and below the median. The mean equal to 0.3547 µm is part of the interval (0.3 - 0.35) µm. Overall, the distribution is normal because the mean, mode and median coincide over the same interval (0.3 - 0.35) µm. The standard deviation = 0.06760084. In addition, the frequency curve has a bell shape. The cumulative frequency curve admits a plateau. Therefore, it can be considered as homogeneous. The values obtained during our observations coincide over the same interval (0.3 - 0.35) µm. Under these conditions, the powder obtained gives a normal distribution [8]. This shows that a single population is characterized by the average particle size. In addition, the particle sizes being between 0.02 and 2000 µm, so our measurements respect Féret's theory. In addition, the powders can be used dry or liquid [7].

Granulometry is a fundamental characteristic, directly related to all unit operations of grinding, separation, mixing and transfer (7). Particle size also plays an important role in the rate of dissolution in vitro and in vivo [9]. According to the law of Noyes and Whitney, the speed of dissolution is proportional to the surface offered to the dissolution, but the more a solid is divided, the larger its surface.

The shape of the particles is often difficult to define, the particles rarely having spherical or regular shapes. The most common shapes are regular, spherical shapes.

For irregular shapes, the result obtained is influenced by the measurement method used. The particle size can be

modified by humidity. It is therefore important to control the humidity of the raw materials before they are used and / or to work in controlled humidity conditions if necessary. The particle size can be modulated by grinding or by controlling the process for the synthesis or production of the material. Control of the parameters of grinders, crystallizers, separators is necessary for control of particlesize.

A measurement of the size distribution of the particles is carried out in order to relate this analysis to a property or a behavior of the powder. When choosing a method for measuring particle size distribution, the end application should always be considered. So, for example, it is important to know whether the powder will be used dry or dispersed in a liquid [7].However, the different methods of measuring particle size distribution often have limitations in use. The constituent particles of a powder are subject to a size distribution which may be unimodal or bimodal. To limit the number of particle size classes, a distribution with classes in geometric progression is preferred. The mean of a distribution is a measure of central tendency. Among the most widely used central trends are the mode, the median and the average. The mode is the most frequent diameter in a distribution; it corresponds to the maximum of the frequency curve. The median represents the value where the total frequency of values above and below are the same (that is, we find the same numberor total particle volume below the median, as well as above). The average must be calculated and it determines the point where the moments of the distribution are equal. For a normal distribution, the mode, the mean and the median coincide; whereas they differ in the case of a non-normal distribution.

The size of the particles is dependent on the spraying equipment (mill; drying, amplitude and agitation times). For different moduli and stirring times, a homogeneous powder can be heterogeneous by sieving; fine particles could be retained on a sieve in the larger particle fractions. Another parameter such as volume should be associated with the characterization. In fact, a very small number of large, poorly pulverized particles could impose their size on all the particles by imposing a higher mass than a large number of large fine particles which could weigh less. Therefore the percentages by weight do not always reflect the correct interpretation characteristic of a particle size. On the other hand, the microscopy method is welcome because for any fraction, it not only allows the measurement and direct observation of the sizes of the particles but also allows them to be reduced to the number of total particles of the fraction used. Thus this method by microscopy seems to be more reliable for the particle size distribution.

5. Conclusion

The micrographic study carried out on the dried bark powder of *Landolphiaowariensis*allowed us to know its constituent elements which characterize a bark of vegetable plants. Therefore it is natural and meets the standards of the French Pharmacopoeia 7 thediting.These elements are made from the structure of the bark itself and brought out by spraying the bark. However, these same elements are found in certain drugs. They would not have too great an impact on the anti hemorrhoidal activity dependent on the chemical composition. This work has enabled us to make a contribution to the biological knowledge of this species. A formulation in liquid or dry form is conceivable.

6. Acknowledgment

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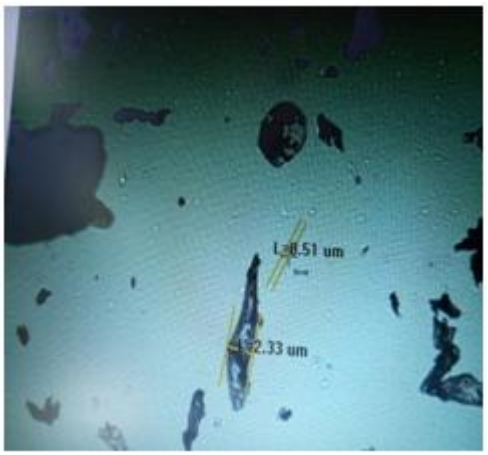
Annex

Granulometric blade with a pinch of powder



Particle size measurement (Féret)

Device for particle size distribution by microscopy and calibration of the particle size slide

Direct observation of sizes on the computer